

The Actions of N-Propyl Ajmaline on Experimental Arrhythmias and Electrophysiological Properties of the Heart

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The antiarrhythmic and general cardiovascular effects and acute toxicity of N-propyl ajmaline (NPA) and ajmaline (AJM) were studied in the dog, rabbit and mouse. NPA was approximately three to seven times more potent than AJM. In addition, the antiarrhythmic mechanism was investigated electrophysiologically on the isolated guinea pig atria and on the isolated dog ventricular muscle, right bundle branch and Purkinje fibres. Both drugs caused a decrease in the spontaneous beating rate of the atria, a prolongation of the refractory period and a reduction in the conduction velocity in the Purkinje fibres. The results of the study suggest that the actions of both drugs do not substantially differ, although they have quantitative differences.

The effectiveness of ajmaline (AJM), one of the rauwolfia alkaloids, against certain experimental and clinical arrhythmias has been well established.²⁾ It has been shown that the antiarrhythmic properties of ajmaline may be attributed to a decrease in the automaticity of the atria, a depression of the conduction in the ventricular tissue and a prolongation of the effective refractory period.³⁾

N-propyl ajmaline (NPA) has been found to be 10 times as potent as quinidine if tested by the prolongation of the effective refractory period in isolated guinea pig atria.⁴⁾ Heistracher, *et al.*⁵⁾ showed that NPA reduced the rate of rise, the amplitude and the overshoot of the action potential, without affecting the resting potential in the Purkinje fibres of calves and sheep.

The present study was undertaken, therefore, to examine the actions of NPA and AJM on the experimental arrhythmias and overall circulatory system and their acute toxicity. Furthermore, the antiarrhythmic mechanisms of NPA and AJM on isolated guinea pig atria, isolated dog ventricular muscle, right bundle branch and Purkinje fibres were investigated from electrophysiological points of view.

Materials and Methods

1. Blood Pressure, Respiration and Electrocardiogram (ECG) in the Anesthetized Dog—Six mongrel dogs of either sex weighing 9.0–11.0 kg were anesthetized with pentobarbital sodium (30 mg/kg, *i.v.*). A tracheal cannula was inserted and respiration was recorded on an ink-writing oscillograph (Nihon Kohden WI-180) through a transducer (Nihon Kohden MFP-1T). Arterial blood pressure was recorded by means of a pressure transducer (Nihon Kohden MP-4T) from the left femoral artery. ECG (lead II) was displayed.

- 1) Location: a) Hongo, Bunkyo-ku, Tokyo, 113, Japan; b) Tanabe-dori Mizuho-ku, Nagoya, 467, Japan.
- 2) R.B. Arora and B.R. Madam, *J. Pharmacol. Exptl. Therap.*, **117**, 62 (1956); H. Schmitt and Mme. H. Schmitt, *Arch. Intern. Pharmacodyn.*, **127**, 163 (1960); E. Hohnloser, *Med. Klin.*, **14**, 550 (1960).
- 3) G. Kuschinsky and H. Reuter, *Arch. Exptl. Pathol. Pharmacol*, **242**, 17 (1961); A. Petter and K. Zipf, *Arch. Exptl. Pathol. Pharmacol*, **243**, 519 (1962).
- 4) R. Kadatz and W. Rossbach, *Arch. Exptl. Path. Pharmacol*, **245**, 7 (1963).
- 5) P. Heistracher and B. Pillat, *Arch. für Kreislaufforschung*, **44**, 300 (1964).

on an electrocardiograph (Nihon Kohden MC-35). PQ, QRS and QT intervals were measured from ECG (lead II). The test drugs were injected into the right femoral vein through a polyethylene cannula.

2. Ouabain-Arrhythmias in the Anesthetized Dogs—Fourteen mongrel dogs of either sex weighing 7.0–12.0 kg were anesthetized with pentobarbital sodium (30 mg/kg, *i.v.*). Blood pressure in the left femoral artery was measured with a pressure transducer. ECG was displayed on an electrocardiograph.

To produce cardiac arrhythmias, an initial dose of 40 μ g/kg of ouabain was administered intravenously. This dose was supplemented 30 min later by a dose of 20 μ g/kg, and every 15 min thereafter by an additional 10 μ g/kg of ouabain until nodal tachycardia or ventricular tachycardia appeared.⁶⁾ The test drugs were administered into femoral vein over periods of 1 to 3 min.

3. Arrhythmias induced by Intracerebroventricular Picrotoxin in the Rabbit—Male rabbits weighing 2.3–2.8 kg were anesthetized with ether. After animals were placed in a stereotaxic instrument (Takahashi B-301), *d*-tubocurarine chloride was injected and artificial respiration was maintained throughout the study. A cannula was inserted into the left lateral ventricle according to the stereotaxic coordinate of Sawyer, *et al.*⁷⁾ Picrotoxin (50 μ g) in a volume of 0.2 ml was injected for 30 sec. The test drugs were administered into the ear vein in 5 to 10 min after the administration of picrotoxin. Arterial blood pressure was recorded on an ink-writing oscillograph from the cannulated femoral artery *via* a pressure transducer and ECG (lead II) was recorded simultaneously.

4. Guinea Pig Atrial Preparation—Male guinea pigs weighing 300–400 g were killed by a blow on the head and the heart was quickly removed. The atrial preparation was made in the oxygenated Tyrode solution and suspended in a 30 ml organ bath containing Tyrode solution. The bath fluid was aerated with 95% O₂+5% CO₂ and maintained at 37°. Isometric tension was recorded on an ink-writing oscillograph through a force-displacement transducer (Nihon Kohden SB-1T). The beating rate was continuously recorded on an ink-writing oscillograph through a pulse rate tachometer (Nihon Kohden RT-2).

5. Intracellular Recording in the Dog Heart—Dogs of either sex weighing 8.0–10.0 kg were anesthetized with thiopental sodium (35 mg/kg, *i.v.*). The heart was quickly removed and right ventricular wall and upper interventricular septum with the right bundle branch were excised. The preparation was fixed on a paraffin block in the organ bath containing 30 ml of a modified Tyrode solution which was circulated by bubbled oxygen and maintained at 37°. The composition of the solution was as follows: NaCl 147.0, KCl 2.7, CaCl₂ 1.8, MgCl₂ 0.49, NaH₂PO₄ 3.0, glucose 5.5 mm.

The transmembrane potential was recorded through a glass microelectrode (3M KCl, 5–10 megohms). The electrical activities of the cell membrane were displayed on a cathode ray oscilloscope (Nihon Kohden VC-7). In some experiments, the preparation was electrically driven with a pair of Ag–AgCl stimulating electrodes.

For the measurement of the conduction velocity, two microelectrodes were impaled into the same Purkinje fibre bundle at an interval of 2 to 3 mm. The stimulating electrode was placed on the fibre bundle 5 to 6 mm apart from the recording electrode. Individual action potential from each microelectrode was displayed on the same oscilloscope at a high sweep and the time interval between the peaks of the action potential was measured. The conduction velocity was calculated from the time interval and the distance between the microelectrodes.

In the investigation of the refractory period in the Purkinje fibre, the strength-interval curve was obtained.

6. Acute Toxicity—Acute toxicity study was carried out with NPA and AJM in ddY-strain male mice weighing 17.0–21.5 g by oral and intravenous routes. Mice were divided into five groups (10 mice per dose) and lethal effects were determined over the period of three days. The lethal dose, 50% (LD₅₀) and associated confidence limits were calculated according to the method of Litchfield and Wilcoxon.⁸⁾

7. Drugs—The following drugs were used; N-propyl ajmaline hydrogentartrate (NPA), ajmaline (Gilurytmal®, Nippon Chemipharm Co., Ltd.), picrotoxin (Tokyo Kasei), quinidine sulfate (Wako Pure Chem. Industries) and g-strophanthin (ouabain, Tokyo Kasei).

Results

1. Blood Pressure, Respiration and ECG in the Anesthetized Dog

The administration of 1.0 mg/kg of AJM produced an increase in blood pressure of only 10 to 15 mmHg for about 5 min (Fig. 1). The high dose of 5 mg/kg produced an immediate and maximum fall in blood pressure of 30 to 60 mmHg and there was a lapse of 60 min before blood pressure returned to the control level. NPA (0.1–0.5 mg/kg) exerted no effect on

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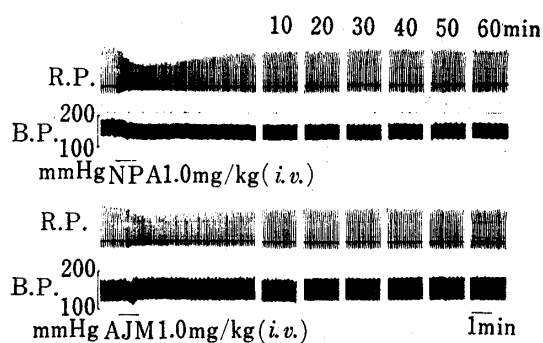


Fig. 1. Effects of AJM and NPA on the Respiration and Blood Pressure in Anesthetized Dogs

R.P.: respiration, B.P.: blood pressure

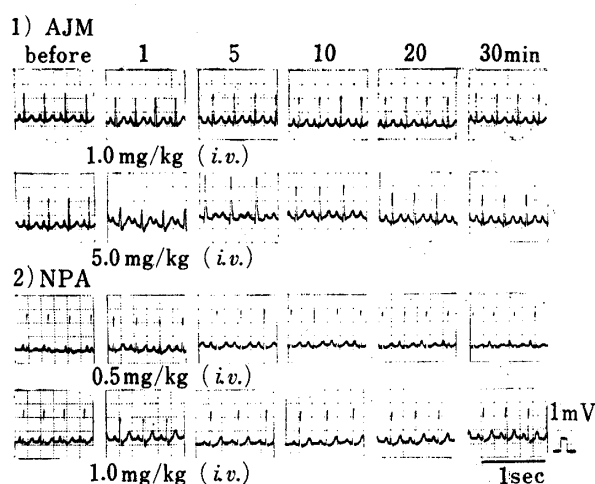


Fig. 2. Effect of AJM and NPA on ECG in Anesthetized Dogs

Table I. Effect of NPA and AJM on ECG (Lead II) in the Dog

Drug	Dose mg/kg	Changes of ECG component (%)		
		PQ-interval	QRS complex	QT-interval.
NPA	0.1	13.0±1.8	4.8±4.7	7.4±1.9
	0.5	58.9±9.2	26.8±6.4	19.0±1.2
	1.0	91.6±6.8	77.7±11.1	39.1±3.5
AJM	1.0	19.7±2.2	22.2±11.1	7.5±1.6
	5.0	75.0±14.4	95.3±19.4	20.7±6.4

Data were obtained 5 min after the injection of drugs. All values represent means in separate 3 experiments, with the S.E. indicated.

blood pressure or at most a slight increase of 5 mmHg. A dose of 1.0 mg/kg produced a fall of 10 to 30 mmHg which lasted longer than 60 min (Fig. 1).

The injection of AJM (1.0—5.0 mg/kg) or NPA (0.5—1.0 mg/kg) produced a slight and transient increase in the respiratory rate, and a transient decrease in the respiratory depth.

The effects of AJM and NPA on the ECG are shown in Table I and Fig. 2. AJM (1.0 mg/kg) produced no change or a transient increase in the heart rate. The dose of 5.0 mg/kg produced a slight decrease in the heart rate accompanied by the elevation of T wave, the decrease in the amplitude of R wave and the prolongation of PQ (75.0%), QRS (95.3%) and QT (20.7%) interval which lasted for 30 to 40 min. The mode of action of NPA was similar to that of AJM, although the effect of NPA was stronger than that of AJM. The dose of 0.1 mg/kg of NPA produced no change in the heart rate, but 1.0 mg/kg produced a decrease in the heart rate, the elevation of T wave, the decrease in the amplitude of R wave and the prolongation of PQ (91.6%), QRS (77.5%) and QT (39.1%) intervals.

2. Ouabain-Arrhythmias in the Anesthetized Dog

Ventricular multifocal extrasystoles or ventricular tachycardia were induced in anesthetized dogs by the intravenous administration of ouabain. In a control series, the arrhythmia was evident for at least 60 min after the injection of saline.

The effects of AJM and NPA on ouabain-arrhythmias are shown in Table II. In one of two dogs, 0.5 mg/kg of NPA converted arrhythmias to normal sinus rhythms. The administration of 1.0 mg/kg caused a sustained conversion to normal sinus rhythms in three out of four dogs. The duration of the conversion lasted for 30 to 40 min. In these cases, the

TABLE II. Antiarrhythmic Action of NPA and AJM on Ouabain-Arrhythmias in Anesthetized Dogs

Drug	Dose (mg/kg)	Ouabain ^{a)} ($\mu\text{g}/\text{kg}$)	Number of animals		Duration (min)	Heart rate (beats/min)		
			Used	Converted ^{b)}		Control	Ouabain	Drug
NPA	0.2	60	2	0	0	163	210	205
	0.5	65	2	1	30	166	215	165
		(60—70)						
1.0	63	4	3	(35—40)	170 \pm 13	227 \pm 14	171 \pm 10	
AJM	1.0	60	3	1	18	183 \pm 10	210 \pm 5	190 \pm 5
	3.0	60	3	2	22.5 (15—30)	175 \pm 11	230 \pm 13	178 \pm 9

a) An initial dose (40 $\mu\text{g}/\text{kg}$) of ouabain was injected *i.v.* This dose was supplemented 30 min later by a dose of 20 $\mu\text{g}/\text{kg}$, and every 15 min thereafter by an additional 10 $\mu\text{g}/\text{kg}$ of ouabain until ventricular arrhythmia appeared. Drugs were injected *i.v.* over periods of 1 to 3 min.

b) Number of animals in which conversion to sinus rhythm occurred. Data represent mean values, with the S.E. indicated and the range is shown in parentheses.

sinus rate returned to the normal level. AJM in a dose of 1.0 mg/kg caused the transient abolition of arrhythmias in one of three dogs. The administration of 3.0 mg/kg was effective in two out of three dogs.

3. Arrhythmias induced by Intracerebroventricular Picrotoxin in the Rabbit

The intracerebroventricular injection of picrotoxin always induced a marked rise in blood pressure (60—100 mmHg) and cardiac arrhythmias such as ventricular extrasystole, ST-T segment deviations and ventricular tachycardia. Arrhythmias had an average latency of 4 min and duration of longer than 30 min.

As shown in Fig. 3, 0.2 mg/kg of AJM converted the cardiac arrhythmias to regular sinus rhythm for 5 to 8 min in two of three experiments. The antiarrhythmic effect of 0.5 mg/kg lasted for longer than 20 min in two of three experiments and for 8 min in one experiment.

The administration of 0.05 mg/kg of NPA was ineffective in improving the arrhythmias. The dose of 0.1—0.2 mg/kg converted the arrhythmias to the regular sinus rhythms for longer than 20 min in 5 experiments and reduced the rise of blood pressure induced by intracerebroventricular picrotoxin.

4. Effects of AJM and NPA on the Guinea Pig Atria

NPA in a concentration of $1 \times 10^{-6} \text{M}$ exerted a moderate negative chronotropic effect on the spontaneously beating guinea pig atria without affecting the contractility. When

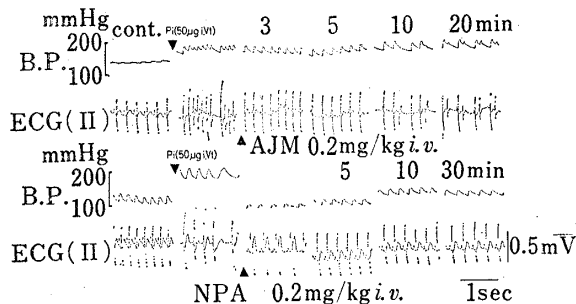


Fig. 3. Antiarrhythmic Action of AJM and NPA on Arrhythmias induced by Intracerebroventricular Picrotoxin

Picrotoxin (Pi) 50 μg in a volume of 0.2 ml was injected for 30 sec intracerebroventricularly (*i. Vt.*). Drugs were administered *i.v.* in 10 min after the administration of picrotoxin.

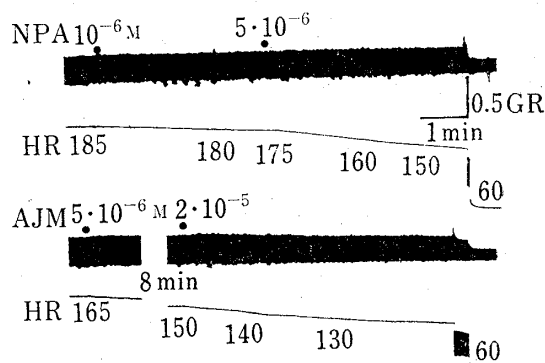


Fig. 4. Effect of AJM and NPA on the Contractility and the Beating Rate of the Guinea Pig Atria

the concentration of NPA was elevated to $3 \times 10^{-6} M$, a remarkable chronotropic effect was observed. A few minutes after an application of the higher dose ($5 \times 10^{-6} M$) of NPA, a sudden fall of the beating rate accompanied by the rapid decrease in the contractile force of the guinea pig atria was usually observed. Some arrhythmic events also appeared during the process of the sudden change in the automaticity. The depressant effect of AJM ($2 \times 10^{-5} M$) on the guinea pig atria was similar to but weaker than that of NPA (Fig. 4).

The time courses of the sudden decrease in the beating rate are shown in Fig. 5. The administration of either $1 \times 10^{-5} M$ AJM or $3 \times 10^{-6} M$ NPA induced the sudden decrease in the automaticity of the guinea pig atria, but the action of NPA appeared earlier than that of AJM.

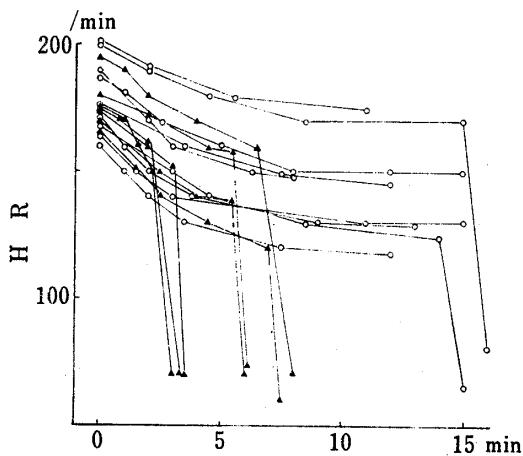


Fig. 5. The Time Course of AJM and NPA on the Beating Rate in Guinea Pig Atria

A sudden fall of the beating rate was accompanied by the rapid decrease in the contractile force and the arrhythmias. H.R.: heart rate

○—○: AJM $10^{-5} M$ ▲—▲: NPA $3 \cdot 10^{-6} M$

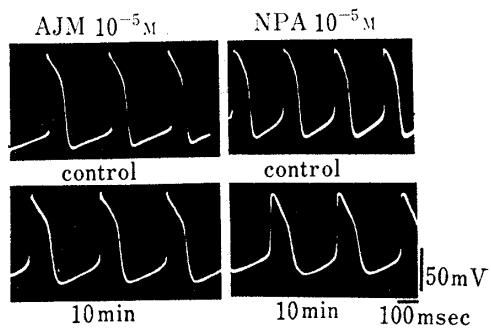


Fig. 6. Effect of AJM and NPA on the Pacemaker Potential of the Dog Right Bundle Branch

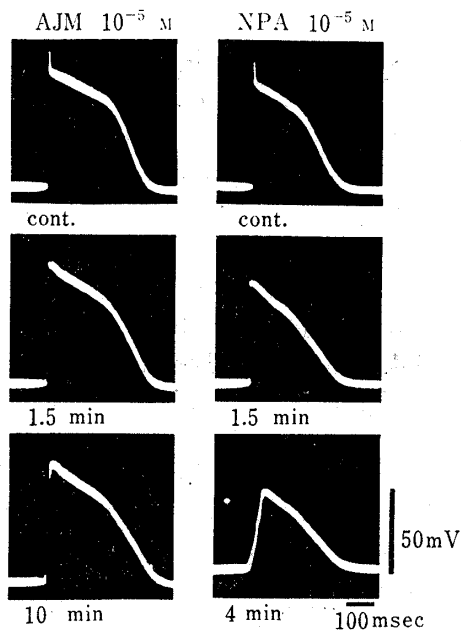


Fig. 7. Effect of AJM and NPA on the Action Potential in the Terminal Purkinje Fibre of the Dog Right Ventricle

The overshoot of spike potential diminished 1.5 min after the application of drugs.

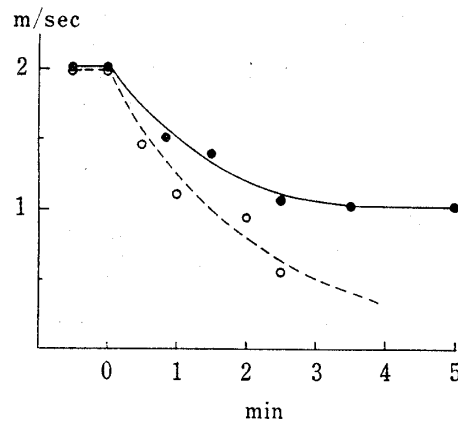


Fig. 8. Effect of AJM and NPA on the Conduction Velocity in the Purkinje Fibre of the Dog

Ordinate represents the conduction velocity. Abscissa represents time after drug application. ●—●: AJM $10^{-5} M$ ○—○: NPA $10^{-5} M$

5. Intracellular Recording in the Dog Heart

5-1. Effect on the Action Potentials of the Right Bundle Branch and Purkinje Fibres of the Dog—Fig. 6 represents the effects of AJM and NPA on the pacemaker potential of the right bundle branch. NPA and AJM reduced the slope of slow diastolic depolarization, the rate of rapid depolarization and the rate of repolarization. All these alterations in the action potentials seemed to contribute to the decrease in the spontaneous beating rate.

AJM and NPA decreased the rate of rise of the action potential in the terminal Purkinje fibre. The overshoot of spike potential disappeared 1.5 min after the application of the drugs (Fig. 7). In all cases, NPA was more potent than AJM.

5-2. Effect on the Conduction Velocity—Both AJM and NPA significantly reduced the conduction velocity in the Purkinje fibres, although NPA was more potent than AJM (Fig. 8).

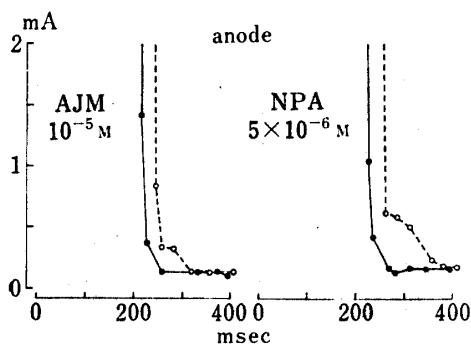


Fig. 9. Strength-Interval Curve for AJM and NPA in the Purkinje Fibre of the Dog

An anodal pulse of 5 msec duration was applied to the preparation through a single Ag-AgCl electrode. Strength was measured in mA and interval in msec. Solid line represents the anodal thresholds in normal Tyrode solution; broken line, the anodal thresholds in the presence of AJM or NPA.

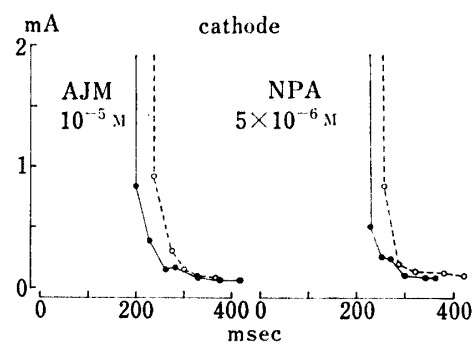


Fig. 10. Strength-Interval Curve for AJM and NPA in the Purkinje Fibre of the Dog

A cathodal pulse of 5 msec duration was applied to the preparation through a single Ag-AgCl electrode. Solid line represents the cathodal thresholds in normal Tyrode solution; broken line, the cathodal thresholds in the presence of AJM or NPA.

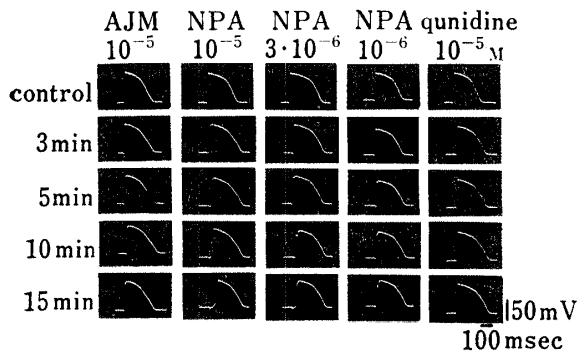


Fig. 11. Effect of AJM, NPA and Quinidine on the P-V Conduction in the Terminal Purkinje Fibre

NPA and AJM produced a notch in the rapid depolarization phase.

(Fig. 11). NPA was the most potent of the three in modifying the configuration of the action potential.

6. Acute Toxicity

The results of LD₅₀ in mice for NPA and AJM are shown in Table III. Intravenous and oral acute LD₅₀ in mice for NPA were 3.4 mg/kg (3.1—3.7 mg/kg) and 65.5 mg/kg (58.5—

5-3. Effect on the Refractory Period

—As shown in Fig. 9 and 10, the strength-interval curve shifted to the right in the presence of AJM (10⁻⁵M) or NPA (5 × 10⁻⁶M), when the shock of either an anodal or cathodal pulse was employed. This indicates that AJM or NPA prolonged the effective refractory period (Fig. 9, 10).

5-4. Effect on Terminal Purkinje Fibres

—AJM, NPA and quinidine reduced the rate of rise and the spike amplitude of the action potential in the terminal Purkinje fibres, and also clearly caused a dissociation of the spike potential and a slow rise of depolarization of the action potential

TABLE III. Acute Toxicity of NPA and AJM in Mice

Drug	LD ₅₀ (mg/kg) and 95% confidence limits	
	Oral	Intravenous
NPA	65.5 (58.5— 73.4)	3.4 (3.1— 3.7)
AJM	350.0 (307.0—399.0)	25.0 (20.0—30.0)

73.4 mg/kg) respectively. Intravenous and oral acute LD₅₀ for AJM were 25.0 mg/kg (20.0—30.0 mg/kg) and 350 mg/kg (307.0—399.0 mg/kg) respectively.

The symptoms caused by NPA and AJM included the decreased activity, prostration, ataxia, ptosis, loss of righting reflex and clonic convulsion with increase in dose. Fatalities were preceded by recurring clonic convulsions appearing within one min and 20 min following intravenous and oral administration of either drug, respectively.

Discussion

AJM has been shown to be effective in the treatment of cardiac arrhythmias.^{2,9)} Philipsborn¹⁰⁾ reported that NPA (a N-propyl derivative of AJM) was five to six times more effective than AJM on aconitine-induced arrhythmia in rats. Recently, Nakayama, *et al.*¹¹⁾ showed that NPA was effective on aconitine-induced atrial and ouabain-induced ventricular arrhythmias in anesthetized dogs. In our study, AJM protected against ouabain-induced arrhythmias in the anesthetized dog and NPA was three times or more as potent as AJM.

Bircher, *et al.*¹²⁾ reported that cardiac arrhythmias induced by intracerebroventricular administration of picrotoxin in the dog were central in origin and were mediated *via* both vagal and sympathetic systems. Intracerebroventricular injection of picrotoxin led to cardiac arrhythmias in the rabbit. It was confirmed that AJM and NPA converted the cardiac arrhythmias to the regular sinus rhythm and NPA was more potent than AJM.

Göing, *et al.*¹³⁾ observed that AJM prolonged the P wave, PQ and QT-interval in the ECG of guinea pig. We also obtained the results that AJM and NPA lengthened PQ, QRS and QT-interval in the ECG of dogs and that NPA was more potent than AJM (Table I).

In the isolated guinea pig atrial preparation, AJM and NPA markedly reduced the spontaneous beating rate without affecting the contractility. In a toxic dose, they showed a negative inotropic effect.

On the pacemaker cells of the dog right bundle branch, AJM and NPA caused a decrease in the slope of the slow diastolic depolarization, a reduction in the rate of rapid depolarization and a prolongation of the repolarization. In the spontaneously beating Purkinje fibres of the calves, AJM was shown to reduce the spontaneous discharge rate and to decrease the rate of rise, the amplitude and the overshoot of the action potential leaving the maximal diastolic potential practically unchanged.¹⁴⁾

In the Purkinje fibres, both NPA and AJM significantly reduced the conduction velocity and prolonged the refractory period. The significant decrease in the conduction velocity seems to be mainly a consequence of the reduction in the rate of rise of the action potential. Heistracher and Pillat^{5,14)} studied the effect of NPA and AJM on the relationship between

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membrane potential and maximal rate of rise of the action potential in the Purkinje fibres of calves and sheep and showed that NPA and AJM reduced the rate of rise of the action potential. It was pointed out that NPA and AJM stabilized the membrane of the Purkinje fibres and reduced the Na conductance, as in the case of quinidine and procaine amide.¹⁵⁾

In the terminal Purkinje fibres, NPA and AJM produced a notch in the rapid depolarization phase and selectively abolished the spike potential. An appearance of a notch in the depolarization phase was previously demonstrated to be detected in the transitional cardiac cells between Purkinje fibres and myocardial cells of the ventricular muscle.^{16,17)} The notch is thought to be due to the different activation time between Purkinje fibres and the ventricular cells adjacent to junctional cells. Therefore, NPA and AJM seem to affect the terminal Purkinje fibres or the transitional cells and to cause a P-V conduction delay. Both drugs abolished the spike potential without affecting the amplitude of the second rise of depolarization. It is generally said that the spike potential is originated in the Purkinje fibres and the second depolarization in the myocardial cell, respectively. This suggests that both drugs suppressed an electrical activity of the terminal Purkinje fibres more specifically than that of myocardial cell.

In our toxicity study, the course of intoxication by NPA and AJM did not substantially differ in mice and the acute toxicity of NPA was five to seven times greater than that of AJM in mice. Weider, *et al.*¹⁸⁾ also observed a similar result in the cat.

In all cases, NPA and AJM showed qualitatively identical action, although NPA exerted stronger actions than AJM. It is suggested that the antiarrhythmic mechanisms of AJM and NPA are based on a suppression of the spontaneous activity of pacemaker cell, a prolongation of the refractory period and a reduction in the conduction velocity of the Purkinje fibres.

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